REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and the following commentary.

I. Status of Claims

Claims 2, 8, and 10 are cancelled without prejudice or disclaimer. Applicant reserves the right to pursue the subject matter of any cancelled claim in a continuing application.

Claim 1 is amended to recite that the targeted gene delivery is effected through degradation of the minicells in late endosomes, whence therapeutic nucleic acid sequences escape, permitting expression of the sequences in mammalian cellular nuclei. Support for the amendment is found throughout the original specification. Thus, the first paragraph on page 10 states that "recombinant plasmid escape from the late-endosome of non-phagocytic cells." The first paragraph on page 12 states that the recombinant DNA "escapes the endosomal membranes and is transported to the mammalian cell nucleus, permitting gene expression."

Claim 1 also is revised to recite a "plurality" of therapeutic nucleic acid sequences, with support in specification, for example, at page 10, second full paragraph. Claims 3, 12, 13, and 16 are likewise amended.

Claim 1 also is revised to state that the recited minicells are approximately 400 nm in diameter. See the application at page 12, second full sentence, at page 31, second full paragraph, and at page 32, second full paragraph.

New claims 36-38 are supported in the second full paragraph on page 10 of the specification, as well as on page 11, lines 2 and 3, for instance.

No impermissible new matter has been introduced. Accordingly, applicant requests entry of this amendment, whereupon claims 1, 3-7, 9, 12-18, and 36-38 will be pending.

II. Response to Sections 102 and 103 Rejections

A. Overview of Response

The examiner has rejected claims for alleged anticipation by Sabbadini *et al.*, U.S. patent No. 7,183,105, and/or for alleged obviousness over Sabbadini and two secondary references.

In response, applicant elaborates on the claimed invention, highlighting certain recited features. Applicant then deconstructs the disclosure of Sabbadini, in order to belie the contention that the reference specifically discloses these features, which would be prerequisite to a sustainable anticipation rejection. Yet, it is clear from this deconstruction that many such features are related by Sabbadini, if at all, only generally, in genus fashion, or in a manner inconsistent with common usage. According to well-established case law and the MPEP, there can be no anticipation when the knowledgeable reader must choose among various meanings and from different permutations of features that a reference is purported to disclose. Only impermissible hindsight, informed applicant's own invention, could allow such choosing from among the scores of permutations thrown off by the Sabbadini text. It necessarily follows, therefore, that Sabbadini does not anticipate the present claims.

Applicant further demonstrates that, in addition to disclosing certain features in genus fashion, Sabbadini also conveys various species in a fungible manner, without relevant guidance, or even in a way that disfavors what applicant's present claims encompass. With this perspective, it is apparent that the teachings of Sabbadini would not have motivated but rather would have disincentivized the skilled artisan to implement the specific aspects of applicant's claimed invention. Accordingly, the cited combination of Sabbadini and the secondary references does not establish a *prima facie* case of obviousness.

Finally, applicant's claimed invention is associated with several significant findings and results that would have been wholly unexpected in light of the prior art represented by the Sabbadini and the scientific literature invoked there. The present response underscores these surprising aspects of the invention by evidencing, for instance, the incredulity of independent experts who evaluated a description of the invention for one of the most prestigious journals in the field. The non-obviousness of the present claims is further buttressed by the surprising quality of applicant's invention, validated by the only experimental data marshaled in the contemporaneous minicell literature, including the Sabbadini reference.

B. The Claimed Invention

The claimed invention is directed to a targeted gene delivery method that brings bispecific ligands, such as antibodies (defined in the specification from page 14, first full paragraph, *et seq.*), into contact with (a) intact, bacterially derived minicells that contain therapeutic nucleic acids and (b) non-phagocytic mammalian cells. In this context, the bispecific ligands have a specificity for a mammalian cell surface receptor, the binding of which activates receptor-mediated endocytosis (rME) targeted for the endosomal/lysosomal compartment, as discussed in detail below (see the specification also on page 12, in the first full paragraph, *inter alia*, and at Examples 1-3). The endocytic pathway thus implicated is depicted schematically below.

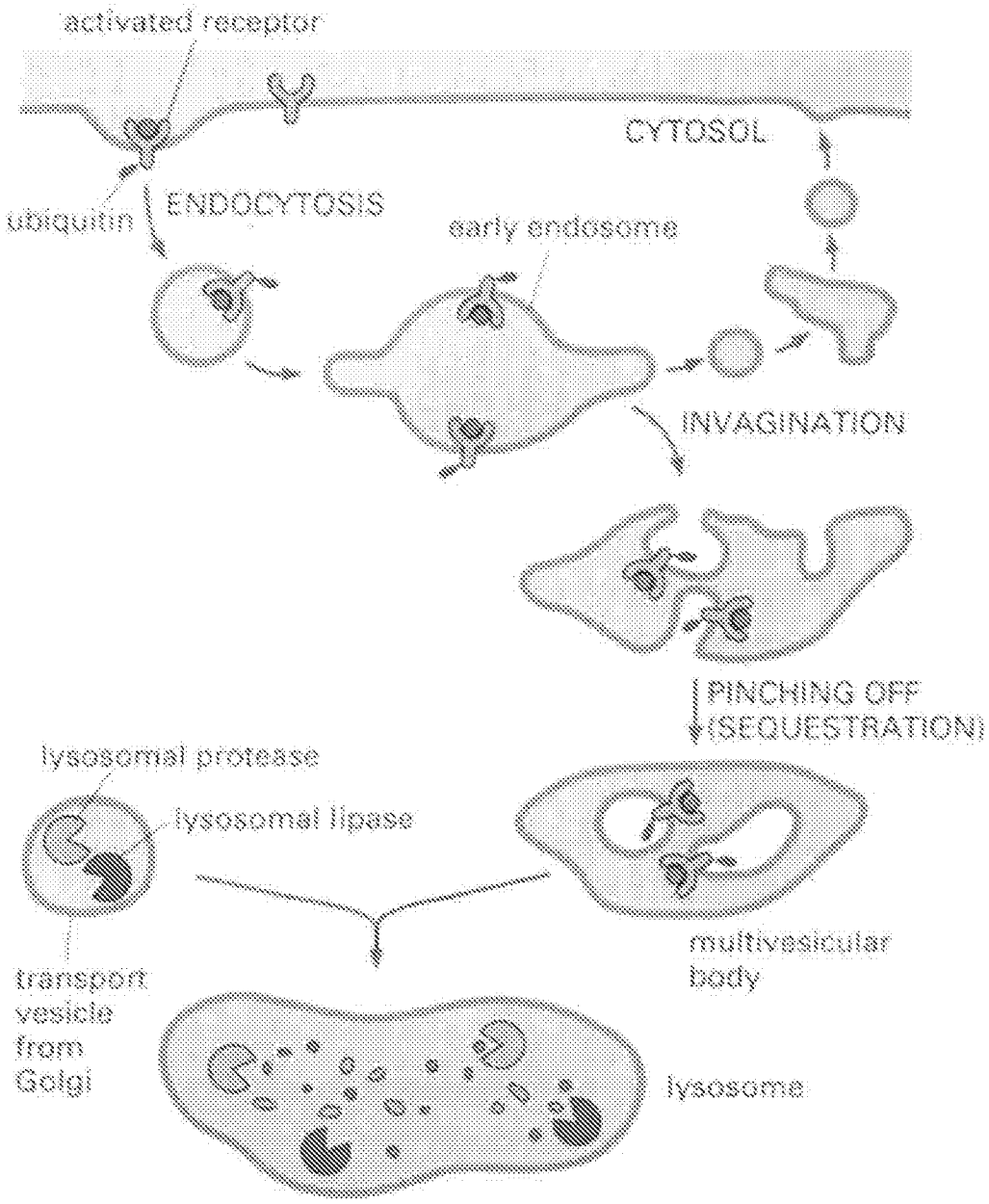
By virtue of such specificity, the bispecific ligands cause the minicells to bind to the non-phagocytic mammalian cells, which consequently engulf the minicells via rME, even though the minicells, approximately 400 nm in diameter, are larger particles than rME was expected to accommodate (see specification, *e.g.*, at page 11, last paragraph). The minicells thereby internalized are subjected to a surprisingly efficient intracellular mechanism that the present inventors were the first to demonstrate, in non-phagocytic cells, could degrade endocytosed particles as large as intact, bacterially derived minicells (see Example 4). By that mechanism the minicells are degraded in late endosomes, as the inventors discovered, releasing the therapeutic nucleic acid sequences. Therapeutic nucleic acid sequences thus escape from late endosomes and are transported to mammalian cellular nuclei, permitting expression there that the inventors have demonstrated can counteract tumors in accepted animal models (see Examples 5 – 7).

1. ***The recited bispecific ligands exhibit dual specificities: a specificity for a mammalian cell surface receptor and, upon binding that receptor, a specificity for activating receptor-mediated endocytosis***

As the claims prescribe, the bispecific ligands have a specificity for a mammalian cell surface receptor. This specificity gives rise to the minicells' ability to "target . . . mammalian host cells" and also causes the minicells to bind to the host cells, because such "host cells normally are resistant to adhesion" (see application at page 9, second paragraph).

Further, to ensure that therapeutic nucleic acids within the minicells are delivered to and expressed in the mammalian host cells, the receptor that the bispecific ligand binds must be able

to activate rME in particular. The "endocytosis" rubric encompasses (1) phagocytosis and (2) pinocytosis, itself a category inclusive of (2a) macropinocytosis, which does not require receptor binding, as well as of (2b) clathrin-mediated endocytosis, (2c) caveolae-mediated endocytosis and (2d) clathrin- / caveolae-independent endocytosis, all of which tend to access the late-endosome/lysosome pathway. See Conner & Schmid, *Nature* 422: 37-44 (2003), especially at page 37 in column 1, at lines 11-18 (copy of this publication, along with copies of other publications invoked in this response, is submitted concurrently with a supplemental information disclosure statement), and the drawing immediately below.



ENDOCYTOSIS VIA THE LATE-ENDOSOMAL / LYSOSOMAL PATHWAY

By contrast, "[i]nvasive bacteria induce their own uptake by non-phagocytic host cells." Veiga & Cossart, *Trends in Cell Biology* 16: 499-504 (2006), at page 499 in column 2, first full paragraph, lines 1 and 2 (copy submitted). One of these uptake mechanisms is the zipper

mechanism, "triggered by the interactions between bacterial surface-exposed proteins and cellular receptors" such as "the *Listeria* proteins InlA and InlB." *Id.*, lines 7-15. Compared to endocytosis, such a bacterium-specific mechanism is an active uptake process through which the bacteria "actively induce their entry into target cells for replication and/or dissemination to other host tissues." Pizzaro-Cerda & Cossart, *Cell* 124: 715-27 (2006), at page 719, column 2, in the first full paragraph (copy submitted). "Invasion can proceed by direct engagement of surface host-cell receptors or by direct translocation of bacterial proteins into the host-cell cytosol that will promote rearrangements of the plasma membrane architecture, inducing pathogen engulfment." *Id.* By contrast, an endocytosis is a passive uptake process.

The InlA and InlB proteins are called "molecular invasion proteins" or "**invasins**." It is recognized, for example, that *Listeria* "evolved two major molecular invasion proteins, referred to ... as invasins: Internalin A (InlA, internalin) and Internalin B (InlB)." Pentecost *et al.*, *PLoS Pathogen* 6: e1000900 (2010), at page 1 in column 1, lines 4-6 (copy submitted).

As the present claims prescribe, the interaction between recited bispecific ligands and mammalian cell surface receptors activates a particular endocytosis pathway, involving rME to the late-endosomal/lysosomal compartment (see above schematic). This is distinct from other endocytosis processes, including one triggered by a bacterial invasin.

2. ***The minicells of applicant's invention are a specific kind of achromosomal cell derivative***

The application defines the phrase "bacterially derived minicell" to connote "anucleate forms of E. coli or other bacterial cells, engendered by a disturbance in the coordination, during binary fission, of cell division with DNA segregation" (page 17, first full paragraph). In this context, an "intact" minicell is one with an intact cell wall (*id.*).

i. The minicells of the current invention do not encompass membrane blebs

As noted, the recitation of "minicells" in the present claims does not embrace all manner of cell derivative that lacks a nucleus or chromosomes. For instance, membrane blebs are anucleate vesicles that, by spontaneous blebbing of bacterial membrane, form in the course of normal bacterial growth. These membrane blebs, however, are not "minicells" within the meaning of the instant application, since, for example, they do not result from cell division.

Accordingly, they are removed from a minicell preparation of the invention, *e.g.*, by way of filtration (see PCT/IB02/04632 at page 41, lines 21-23, incorporated by reference into the specification at page 27, line 2).

ii. The minicells of the claimed invention are approximately 400 nm in diameter

Claim 1 prescribes a diameter of approximately 400 nm for the intact, bacterially derived minicells employed in accordance with applicant's invention. This prescription manifests the inventors discovery that, unexpectedly, such minicells display a uniformity in size, *i.e.*, about 0.4 μm, as well as in shape.

By the effective date of this application, it was well-recognized "dogma that particles with a diameter larger tha[n] 120nm cannot enter cells by clathrin-mediated endocytosis[1]." Veiga & Cossart (2006), *supra*, at page 499 in column 2, lines 14-16.

Reference [1] of Veiga & Cossart (2006) is a review article published, only months before applicant's priority date, in a prestigious research journals. Conner & Schmid (2003), *supra*. The Conner/Schmid review notes in particular that the size limitation for caveolin-mediated endocytosis is about 60 nm and for clathrin- and caveolin-independent endocytosis is about 90 nm (see Figure 1 of the publication).

Therefore, the conventional wisdom before the present invention held that rME, with a capacity for particles only about 120 nm or less in diameter, could not accommodate a minicell, which, pursuant to applicant's usage of that term, is approximately 400 nm in diameter.

iii. The minicells of the present claims have intact cell walls

As prescribed by the present claims, the minicells for the targeted gene delivery method are "intact," *i.e.*, are possessing of an intact cell wall. By contrast, applicant notes that minicells without cell walls or cell membranes have also been in the art.

3. The minicells' specific destiny – engulfed by the non-phagocytic mammalian cells, degraded in late endosomes and release therapeutic nucleic acid sequences

The present claims prescribe that the minicells are engulfed by the non-phagocytic mammalian cells through a rME process. As explained above, the conventional wisdom held that a minicell of the claimed invention, with a large diameter of about 400 nm, could not be engulfed via rME. Therefore, the claimed invention is characterized by an unexpected result.

The claims further relate that the minicells are degraded in the late endosomes. As provided above, invasive bacteria can enter a non-phagocytic mammalian by their own invasive uptake mechanisms such as the zipper mechanism, which is mediated by proteins that function as invasins. The bacterium-specific mechanisms, however, are used by the bacteria to exploit the mammalian cellular system and protect themselves within the cells; hence, these mechanisms would not lead to degradation of the bacteria. With this perspective, even were an intact, bacterially derived minicell to enter a mammalian cell via such bacterium-specific mechanisms, the minicell would not be degraded to release the therapeutic nucleic acid content, as the present claims require.

4. The therapeutic nucleic acid sequences survive degradation in late endosomes, escape from the late endosomes and are transported to and expressed in mammalian cellular nuclei

Finally, the present claims entail that the therapeutic nucleic acid released from the minicells survive degradation in the late endosomes and escape from the late endosomes. This manifests another unexpected nature of the claimed invention.

As further explained below, the conventional wisdom, even as provided in the art cited by the examiner, held that nucleic acid could not escape late mammalian cellular endosomes to avoid degradation and effect expression in the mammalian cellular nuclei.

C. No Anticipation by Sabbadini Under Section 102

The examiner rejects claims 1-4, 7-11, and 13-18 for alleged anticipation by the Sabbadini reference, discussed above. Claims 2, 8, and 10 have been cancelled. Applicant traverses rejection of the remaining claims.

1. Requirements of "anticipating" reference

F or a reference to be anticipatory, the "identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989), *cited in* MPEP § 2131. Accordingly, there can be no anticipation when the knowledgeable reader must choose among different permutations of features encompassed, in genus fashion, by the cited reference.

2. Prerequisite for selecting among Sabbadini's various categories precludes meeting requirements for anticipation

As provided in above section describing the claimed invention, the present claims prescribe a bispecific ligand that has dual specificity, *i.e.*, a specificity for a mammalian cell surface receptor and a specificity for activating rME. As demonstrated in detail below, however, Sabbadini discloses a bispecific ligand that induces endocytosis, a genus that embraces not only rME but also non-rME endocytic processes such as phagocytosis and macropinocytosis. In some other embodiments, Sabbadini discloses a bispecific ligand that has a specificity for a mammalian cell surface receptor and requires another ligand, preferably a bacterial invasin, to induce cellular uptake.

Also clear from the above description of the claimed invention, the minicells prescribed by the present claims do not encompass membrane blebs, are uniform in size, and have intact cell walls. Again, Sabbadini's "minicells" constitute a genus that is characterized by a large range of particle sizes and that includes not only membrane blebs but also derivatives denuded of cell walls, a "preferred" embodiment for Sabbadini.

Further, the present claims prescribe a specific delivery path for the minicell enclosed therapeutic nucleic acid sequences which includes uptake of the minicells, degradation of the minicells in late endosomes, release of the nucleic acids from the degraded minicells, escape of the nucleic acids from the late endosomes and transport of the nucleic acids to cellular nucleic leading to expression of the nucleic acids. By contrast, at the minimum, Sabbadini does not disclose such a specific path.

2.1 Sabbadini does not teach a bispecific ligand that has a specificity for a mammalian cell surface receptor and that activates rME leading to the late-endosomal compartment

In rejecting the claimed invention, Examiner Singh interprets "bispecific ligand" to encompass "an antibody that binds to a ligand specific to a minicell as well as receptor on to the mammalian cell surface" (action at page 4, lines 7-8). In this context, the examiner contends that Sabbadini discloses a bacterial minicell "coated with an antibody as a binding moiety that specifically binds a ligand present on the surface of said mammalian cell" and "that the receptor/ligand interaction will result in the endocytosis of the minicell into the target cell wherein the minicell would release and deliver the genetic material (see column 164, lines 28-37)" (*id.* at page 4, lines 11-19).

i. Sabbadini does not teach a bispecific ligand having a specificity for activating rME

The rejection thus invokes the Sabbadini text at column 164, lines 28-37, which is excerpted below.

> Another non-limiting example of gene delivery or transfection using minicells involves the use of ligands to induce receptor mediated endocytosis. By way of non-limiting example, the ligand is expressed on the surface of the minicell, or is attached to the surface of the minicell. A minicell containing genetic material is then able to associate with a target cell expressing the target receptor for the ligand. The receptor/ligand interaction will result in the endocytosis of the minicell into the target cell where the minicell would release and deliver the genetic material.

(Emphasis added.) According to the examiner, this passage teaches an antibody that is equivalent to the "bispecific ligand" of the claimed invention. Applicant respectfully disagrees.

This passage from Sabbadini does not disclose a bispecific ligand that has a specificity for a mammalian cell surface receptor, as the present claims prescribe. Applicant has noted above that such a specificity gives rise to ability of intact, bacterially derived minicells, as recited, to target mammalian host cells. By contrast, the ligands of the Sabbadini passage serve only to enable association between the minicells and the receptor-expressing cells, without reference to how the minicells are targeted to the receptor-expressing cells.

As discussed above, moreover, "endocytosis" is not coincident with rME; rather, it is a category that embraces phagocytosis and pinocytosis. Pinocytosis, in turn, not only includes rME, such as clathrin-mediated endocytosis and caveolae-mediated endocytosis, but also includes macropinocytosis, which does not require receptor binding. This genus is even recognized in the Sabbadini reference itself:

> A minicell containing genetic material may be to a target cell by methods including, but not limited to, **receptor mediated endocytosis**, **cell fusion**, or **phagocytosis** (Aderem et al., Mechanism of Phagocytosis in Macrophages, Annu. Rev. Immunol. 17:593-623, 1999). The minicell gene delivery system is used to deliver genetic material in culture for research applications as well as to cells in vivo as part of gene therapy or other therapeutic applications.

Id. at column 159, lines 4-11 (emphasis added). So informed, the knowledgeable reader would not find in Sabbadini a specific disclosure of a "bispecific ligand," as recited.

ii. Sabbadini teaches that a ligand that is separate from the bispecific ligand is required for activating cellular uptake

The concepts of bispecific ligands and rME, applicant submits, only appears by name in two passages of Sabbadini, both excerpted below.

> Fusion proteins expressed in minicells are used for cancer therapy. In a non-limiting example, phage display antibody libraries are used to clone single chain antibodies against tumor-associated (tumor-specific) antigens, such as MUCH-1 [*viz.* MUC1] or EGFvIII [*viz.* EGFRvIII]. Fusion proteins expressing these antibodies, and further comprising a single-pass transmembrane domain of an integral membrane protein, are used to "present" the antibody to the surface of the minicells. Injected minicells coated with anti-tumor antibodies target the tumor and deliver pro-apoptotic genes or other toxic substances to the tumor. The minicells are engulfed by the tumor cells by processes such [as] **receptor-mediated endocytosis** (by, e.g., macrophages). By way of non-limiting example, toxR-**invasin** could be expressed on the surfaces of the minicells to promote **endocytosis** through the interaction between **invasin** and beta2-integrins on the surfaces of the target cells.

Id. at column 171, line 54 to column 172, line 3 (emphasis added).

> To provide specificity, [b]y way of non-limiting example, the ligand portion of epidermal growth factor (EGF) may be fused to the

> transmembrane domain of ToxR, thus creating a protein that will interact with cells displaying the EGF receptor (EGFR). Likewise, tumor necrosis factor (TNF) may also serve this purpose by stimulating cell-cell interactions between minicells displaying TNF and cells displaying TNF receptor (TNFR). **Although EGF-EGFR and TNF-TNFR interactions may stimulate** cell-cell fusion between minicells and recipient cells, or **minicell uptake, this alone may not be sufficient to efficiently transfer genetic information from minicells. Therefore, a genetic approach to increasing the cell-cell genetic transfer may be** the development of a genetic switch that senses the specificity interaction, e.g. EGF-EGFR interaction, and turns on the **production of a second gene product, e.g. invasin, that stimulates the endocytic event.** By way of non-limiting example, this genetic switch may be similar to the GPCR-G-protein interaction reporter system above, in that an extracellular event stimulates the dimerization of a transcriptional active regulator, thus turning on the production of invasin or invasin-like protein. In either approach, the display system to stimulate transfer of genetic information from minicells to recipient cells may also be applicable to the transfer of substances other than genetic information, e.g. pre-synthesized therapeutic drugs.

Id. at column 252, lines 4-30 (emphasis added).

These passages describe tumor cell surface receptors, e.g., "MUCH-1" [*sic*], EGFR and TNFR, said to provide specificity for a minicell to target a target cell. Meanwhile, both passages clearly indicate that these tumor-specific cell surface receptors are not sufficient to induce uptake of the minicell by the target cell. To stimulate the uptake, in other words, Sabbadini counsels the need for production of a second gene product, e.g., an invasin.

With this perspective, the skilled artisan would have understood Sabbadini to teach a targeting functionality, on the one hand, that relies on tumor-specific (antibody-like) interactions, and a distinct uptake functionality on the other hand, which is provided by a separate factor, such as an invasin. Contrary to the examiner's contention, Sabbadini would not have conveyed to the knowledgeable reader an operational notion of an antibody that targets a target cell receptor **and** that interacts with the receptor to effect endocytosis of the minicell by the target cell.

iii. Sabbadini's uptake-inducing ligand activates an invasive process that is different from rME targeting the late endosome

Sabbadini repeatedly relies on the invasin/beta2-integrin mechanism. In addition to the two passages reproduced above, Sabbadini states,

> [b]y way of non-limiting example, [that] a minicell may express a protein such as **invasin** to induce **receptor mediated endocytosis** (Pepe et al., Yersinia enterocolitica **invasin**: A primary role in the initiation of infection, Proc. Natl. Acad. Sci. U.S.A. 90:6473 6477, 1993; Alrutz et al., Involvement of focal adhesion kinase in **invasin**-mediated uptake, Proc. Natl. Acad. Sci. U.S.A. 95:13658 13663, 1998). **Invasin** interacts with the Beta2 Integrin protein and causes it to dimerize. Upon dimerization the Beta2 Integrin signals for an endocytotic event. Thus a minicell expressing the **invasin** protein will be taken up by cells expressing Beta2 Integrin via **endocytosis**.

Id. at column 163, lines 22-33 (emphasis added).

As noted above, invains such as InlA and InlB induce uptake via the zipper mechanism, which is wholly dissimilar from an activation of rME that, as presently recited, leads to delivery of the minicell, with its therapeutic contents, to the late-endosomal degradation pathway. By invoking invasin in this manner, therefore, Sabbadini actually teaches away from the presently claimed invention. This teaching-away is exacerbated by the lack of guidance in Sabbadini on how rME, as opposed to phagocytosis or the invasive bacterium-specific mechanisms, might be implemented in a therapeutically useful manner.

2.2 Sabbadini's minicells are disclosed in a genus fashion

Also clear from the above description of the claimed invention, the minicells prescribed by the present claims do not encompass membrane blebs, are uniform in size, and have intact cell walls. By contrast, Sabbadini's "minicells" may be any and all manner of cell derivatives that lack chromosomal DNA (see column 38, lines 52-58). Sabbadini's minicells thus embrace a large category that includes membrane blebs, has a large range of sizes, and can be without cell walls.

First, the minicells of the claimed invention do not embrace all manner of cell derivative that lacks a nucleus and specifically do not embrace membrane blebs because membrane blebs

do not result from cell division. These membrane blebs, however, fall into the definition of Sabbadini's minicell as they lack chromosomal DNA.

Second, applicant has demonstrated above that the "minicells" prescribed by the present claims are approximately 400 nm in diameter. By contrast, Sabbadini's "minicell" can have any size from about 0.005, 0.1, 0.15, 0.2 to about 0.25, 0.3, 0.35, 0.4, 045 or 0.5 micrometers (column 38, lines 30 and 31). Thus, Sabbadini's use of "minicell" embraces a genus of cell derivatives that is not coincident with the meaning of "minicell" lent the present claims by applicant's specification.

Moreover, the present claims prescribe that the minicells have intact cell walls. On the contrary, according to Sabbadini, a minicell can be (i) a minicell that has an intact cell wall enclosed in an intact outer membrane, (ii) a poroplast that has the outer membrane removed, (iii) a spheroplast that has disrupted outer membrane and/or cell wall and (iv) a protoplast that has both outer membrane and cell wall removed (*id.* at column 111-113).

Finally, Sabbadini specifically discloses that "the term 'minicell' encompasses derivatives of eubacterial cells that lack a chromosome; derivatives of archeabacterial cells that lack their chromosome(s) … and anucleate derivatives of eukaryotic cells." *Id.* at col. 38, line 62 to col. 39, line 1. The present claims, however, relate to bacterial minicells only.

In summary, Sabbadini discloses "minicell" in broad, genus fashion, inclusive not only of membrane blebs, which are much smaller than 400 nm, but also of "minicells" that lack intact cell walls or "minicells" derived from sources other than bacteria. Only impermissible hindsight could have prompted the skilled artisan to extract, from Sabbadini's genus, bacterially derived and intact minicells, which the present inventors discovered are uniformly about 400 nm in diameter.

2.3 Sabbadini does not disclose a specific pathway for the minicell based targeted gene delivery

Finally, the present claims prescribe a specific delivery path for the minicell enclosed therapeutic nucleic acid sequences which includes (1) uptake of the minicells, (2) degradation of the minicells in late endosomes, (3) release of the nucleic acids from the degraded minicells, (4)

escape of the nucleic acids from the late endosomes and (5) transport of the nucleic acids to cellular nucleic leading to expression of the nucleic acids. At the minimum, Sabbadini does not disclose such a specific path.

In some embodiments, as provided above, Sabbadini teaches a bispecific ligand that is allegedly able to activate "endocytosis." As endocytosis encompasses a diverse category of uptake mechanisms used by both professional phagocytic cells and non-phagocytic cells, this disclosure, even at the maximum, does not teach a specific pathway for targeted gene delivery.

In other embodiments that concern bispecific ligands, which also are discussed above, Sabbadini teaches a separate ligand, exemplified by bacterial invasins, to induce uptake by the target cell. This is a different method from the claimed invention because the invasins are separate from bispecific ligands. Even if this were considered relevant, *arguendo*, such teaching would not convey to the knowledgeable reader what path the minicells would take inside the target cell, since it is recognized that invasins induce an invasive process distinct from rME. An invasive process, by its very nature, would not allow enclosure of the invading particle in late endosomes, let alone degradation in the late endosomes. Again, Sabbadini does not specifically teach a recited feature of the present claims.

The examiner contends that Sabbadini discloses that the minicell-based delivery method "results in transfer of the molecule from the interior of a minicell into the cytoplasm of the target cell (see column 24, line 22, column 165, lines 5-10)." *Id.* at page 7, lines 21-22. The reference does not disclose, however, that such a molecule can be a nucleic acid.

> In one embodiment, the nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein the ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of **a biologically active compound** contained within or displayed by the minicell. In one embodiment, the membrane of the minicell comprises a system for transferring **a molecule** from the interior of a minicell into the cytoplasm of the cell. In one embodiment, the system for transferring a molecule from the interior of a minicell into the cytoplasm of the cell is a Type III secretion system.

Sabbadini at column 24, line 12-23 (emphasis added).

> After delivery to and entry into a targeted cell, a minicell may be designed so as to be degraded, thereby releasing the **therapeutic agent** it encapsulates into the cytoplasm of the cell. The minicell and/or therapeutic agent may include one

> or more organellar delivery elements, which targets a **protein** into or out of a specific organelle or organelles.

Id. at column 165, lines 6-11 (emphasis added).

Sabbadini thus would be understood to disclose only that a biologically active compound or a protein can be transported to the cytoplasm. Accordingly, Sabbadini does not disclose that a nucleic acid, which is much larger than both a compound and a protein, can escape from the late endosome/lysosome of the mammalian cell, as the present claims prescribe.

According to MPEP, there can be no anticipation when the knowledgeable reader must choose among different permutations of features encompassed, in genus fashion, by the cited reference. Here, Sabbadini does not convey to the interested public the presently recited combination of bispecific ligands and intact, bacterially derived minicells with rME leading to the late endosome. Instead, the skilled artisan would have had to pick and choose from among different genera, which might or might not encompass the relevant features, among many others, in order to arrive in theory at applicant's claimed invention. Since only impermissible knowledge of applicant's own invention could guide such choosing, the asserted rationale for an anticipation rejection must fail, warranting its withdrawal.

D. No *Prima Facie* Case under Section 103

The examiner rejects claims 1, 3, 5, and 6 over Sabbadini in view of Nettlebeck *et al.*, *Mol. Ther.* 3: 882-91 (2001), and Coldwell *et al.*, *J. Immunol.* 133: 950-57 (1984). Applicant traverses the rejection.

Sabbadini's disclosures, in genus fashion, are discussed above. In particular, applicant has underscored Sabbadini's disclosure of separate ligands for host-targeting and for activating "endocytosis," preferably via an invasin-mediated zipper mechanism that is distinct from the presently recited rME mechanism. Indeed, Sabbadini repeatedly relies on the invasin/beta2-integrin mechanism for uptake in principle (the reference affords no experimental data of any sort), thereby teaching away from applicant's claimed invention. Even where Sabbadini's speaks of "endocytosis" *per se*, the only contents of "minicells" also mentioned are "compounds and proteins," with no suggestion of a therapeutic nucleic acid, as presently recited.

With respect to the disclosed "minicell" genus, moreover, Sabbadini expressly teaches that minicells should be treated to "denude" them, in order to mitigate a perceived *in vivo* antigenic potential (column 114, lines 9-13). Denuded minicells are ***not*** intact (*Id.*, lines 13 and 14). In this regard Sabbadini states that

> L-form bacterial strains may be used to prepare minicells and are preferred in some embodiments of the invention. L-form bacterial strains are mutant or variant strains, or eubacteria that have been subject to certain conditions, that lack an outer membrane, a cell wall, a periplasmic space and extracellular proteases. Thus, in L-form Eubacteria, the cytoplasmic membrane is the only barrier between the cytoplasm and its surrounding environment.

(*Id.*, lines 51-58; emphasis added.) According to Sabbadini, denuded minicells are preferred over intact ones, once again leading away from applicant's claimed invention.

Read in whole, therefore, the Sabbadini reference would have presented the skilled artisan with a pastiche of possible features, to be selected somehow from various permutations of genera. This selection would have had to be made with no experimental data or other guidance or, as in the case of the "minicell" and ligand/uptake categories, with guidance that led away from the presently claimed invention. Certainly, nothing in Sabbadini would have countered the conventional wisdom, *contra* applicant's claimed invention, that particles larger than 120 nm would be unable to enter a non-phagocytic mammalian cell via an rME process. Thus, the skilled artisan would have been disincentivized rather than motivated to attempt, with a reasonable *a priori* expectation of success, a methodology that entailed invoking a rME-based process to effect uptake of intact, bacterially derived minicells, which are approximately 400 nm in diameter.

According to the examiner, Nettelback teaches "a recombinant antibody as a molecular bridge" (action at page 10, lines 9-10), and Coldwell teaches "production of monoclonal antibodies to antigenic determinants of the O-polysaccharide of … LPS" (*id.* at page 10, lines 21-23). Even as read by the examiner, therefore, nothing plausibly gleaned from these secondary references could have altered the skilled artisan's interpretation of Sabbadini, as described above.

In light of the foregoing, it is apparent that the combination of Sabbadini with the secondary references does not establish a *prima facie* case of obviousness against applicant's claimed invention. Additionally, the prior art of record does not even hint at any criticality attaching, in the present context, to the number of nucleic acid molecules contained in intact,

bacterially derived minicells. By contrast, see the application at page 10, second and third paragraphs. With respect to the deficiency of the asserted *prima facie* case, therefore, claims 36 and 37 are separately patentable.

E. Unexpected Aspect of the Invention Underscore Non-Obviousness

1. It was unexpected that the minicells were engulfed by non-phagocytic mammalian cells

In the response to the Office action mailed September 17, 2009, applicant provided evidence to illuminate the contemporaneous expectation in the field particles as large as an intact, bacterially derived minicells could not passively enter non-phagocytic mammalian cells via receptor-mediated endocytosis (pages 8 and 9, section B). This notion is underscored in the discussion above of the claimed invention. Hence, the skilled artisan would not have expected rME of intact, bacterially derived minicells by non-phagocytic mammalian cells.

In response Examiner Singh contends that Sabbadini teaches that "minicells are engulfed by the tumor cell by receptor mediated endocytosis, thereby releasing toxic drug into the tumor cell (see column 171, column 1, line 62-65)." Action at page 7, lines 18-20. This passage of Sabbadini, as discussed above, relies on the invasin/beta2-integin interaction to effect uptake. Applicant has demonstrated, however, that the invasin/beta2-integin interaction mediates an active invasion process that is wholly distinct from a passive, receptor-mediated endocytosis process as the claimed invention prescribes.

The examiner further contends that "the features upon which applicant relies (i.e., size of minicell diameter) are not recited in the rejected claims" (*id.* at page 8, lines 4-5). Without acquiescing to this apparent rationale for rejection, applicant have amended claim 1 to recite a diameter of approximately 400 for the intact, bacterially derived minicells.

As noted above, it was accepted dogma before applicant's invention that particles as large as intact, bacterially derived minicells would be unable to enter non-phagocytic mammalian cells via rME. The inventors discovered otherwise, however.

That this aspect of the invention would have surprised the skilled artisan is evident by the *actual* reception it received by experts in the field. When confronted with the inventors' objective demonstration of minicell-mediated delivery, as presently recited, experts engaged for

peer review by *Nature Biotechnology* commented on convincing quality of that demonstration and on the prospect of initial skepticism by some in the field. Thus, one reviewer commented that

> [t]his is a very interesting manuscript describing a novel technology for encapsulation and tumor targeted delivery of various chemotherapeutic agents. The authors show **convincingly** that the particles can be loaded with doxorubicin, paclitaxel or cisplatin, and they show extensive in vivo therapy data in which the antitumor activity of drug-loaded minicells is determined by the specificity of an antibody displayed on their surface. Biodistribution studies conducted in nude mice carrying EGFR positive tumor xenografts demonstrated 30% localization of the administered doxorubicin in the tumor at the six-hour time point.
>
> The paper is certainly interesting and falls within the scope of Nature Biotechnology, **but may generate skepticism** since it flies in the face of what is known about the in vivo behavior characteristics of particles of this size.

Another reviewer agreed, noting that

> [t]his is an intriguing report of a new drug delivery system. While the technology is unique and the results appear **impressive**, I share many of the same concerns as the prior reviewers. While some of these issues are minor (400 nM is not a nanoparticle, which is usually reserved for materials less than 100 nM), the ability of particles this large to escape the vasculature and be effectively internalized by receptor-mediated endocytosis **is hard to believe**.

The manuscript in question was published in another prestigious journal. See MacDiarmid *et al.*, *Cancer Cell* 11: 431 (2007) (copy submitted).

The comments of the reviewers manifest an attitude on the part of experts that is incompatible with the examiner's assertion of obviousness. Those experts not only found the minicell-mediated delivery "impressively" validated with actual data, a commodity notably lacking in the art illustrated by Sabbadini, but they also noted the prospect of "skepticism" over the present inventors' showing that minicell "particles this large [can] escape the vasculature and be effectively internalized by receptor-mediated endocytosis," contrary to the conventional wisdom.

2. ***It was unexpected that the therapeutic nucleic acid sequences escape from late endosomes and are transported to mammalian cellular nuclei***

Sabbadini cites numerous technical publications to describe the process of receptor-mediated endocytosis.

> Some targeting strategies make use of cellular receptors and their natural ligands in whole or in part. See, for example, Cristiano et al., "Strategies to Accomplish Gene Delivery Via the **Receptor-Mediated Endocytosis** Pathway," Cancer Gene Ther., Vol. 3, No. 1, pp. 49-57, January February 1996.; S. C. Philips, "Receptor-Mediated DNA Delivery Approaches to Human Gene Therapy," Biologicals, Vol. 23, No. 1, pp. 13 6, March 1995; Michael et al., "Strategies to Achieve Targeted Gene Delivery Via the **Receptor-Mediated Endocytosis** Pathway," Gene Ther., Vol. 1, No. 4, pp. 223-32, July 1994; Lin et al., "Antiangiogenic Gene Therapy Targeting The Endothelium-Specific Receptor Tyrosine Kinase Tie2," Proc. Natl. Acad. Sci., USA, Vol. 93, pp. 8829 8834, 1998. Sudimack et al., "Targeted Drug Delivery Via the Folate Receptor," Adv. Drug Deliv., pp. 147-62, March 2000; Fan et al., "Therapeutic Application of Anti-Growth Factor Receptor Antibodies," Curr. Opio Oncol., Vol. 10, No. 1, pp. 67-73, January 1998; Wadhwa et al., "Receptor Mediated Glycotargeting," J. Drug Target, Vol. 3, No. 2, pp. 111-27, 1995; Perales et al., "An Evaluation of Receptor-Mediated Gene Transfer Using Synthetic DNA-Ligand Complexes," Eur. J. Biochem, Vol. 1, No 2, pp. 226, 255 66, December 1994; Smith et al., "Hepatocyte-Directed Gene Delivery by **Receptor-Mediated Endocytosis**," Semin Liver Dis., Vol. 19, No. 1, pp. 83-92, 1999.

Column 166, lines 31-55 (emphasis added).

With the present response applicant submits a chronological series of three publications, cited by Sabbadini above, each with a title referencing rME: Michael *et al.* (1994), Cristiano *et al.* (1996), and Smith et al. (1999). This series illustrates the contemporaneous art to which Sabbadini, by analogy, directed the knowledge reader for minicell-targeting approaches. All three publications are review articles, relating *in vivo* gene-transfer strategies that employ a construct comprised of a ligand that can be internalized, via rME, by the cell type of interest.

Applicant notes that this approach in principle "confer[s] specificity at the point of vector-cell contact" (Smith *et al.* (1999) at page 84, left column). Smith *et al.* (1999) discusses "liver-directed internalization" effected by targeting the asialoglycoprotein receptor (AsGPr), which "is expressed selectively on hepatocytes" (*id.*, right column). Michael *et al.* (1994) and Cristiano *et al.* (1996) are more general in their comments, but all three groups of authors underscore how "[d]estruction within lysosomes poses a severe threat to the efficacy of gene

delivery by" rME (Smith *et al.* at page 85, left column). See also Michael *et al.* at 225 (Fig. 1), which states that "[e]scape from the cell vesicle system is achieved by [only] a fraction of the internalized conjugate-DNA complex to achieve nuclear localization where heterologous gene expression is achieved."

Therefore, Sabbadini's own disclosure indicates that it would have been unexpected that therapeutic nucleic acid sequences could escape from the late endosomes and be transported to mammalian cellular nuclei. This surprising aspect of the claimed invention was highlighted as well in applicant's previous response, at pages 11 and 12 (section A). There it was noted that, after being internalized by rME, gene delivery vectors are enclosed within endosomal or lysosomal membranes and are therefore separated from the cytoplasm. This constitutes a significant impediment to gene delivery, especially because endosomal and lysosomal compartments can become highly caustic and degrade more than 99% of nucleic acids in a vector. Successful gene delivery vectors, such as viruses and liposomal vectors, have mechanisms that allow nucleic acids to enter the cytoplasm, but skilled artisans would not expect minicells to have such mechanisms.

Accordingly, the skilled artisan had no basis to have expected the contents of intact, bacterially derived minicells to avoid degradation and escape encompassing lysosomes. Rather, the art suggested that highly sophisticated mechanisms are necessary to allow a vector's contents to escape the lysosomal membrane. Notably, minicells are non-living particles and do not possess any lysosomal membrane-destabilizing functions.

In response, the examiner contends that applicant's argument relating to "viruses and liposome for breaching endosomal membrane is irrelevant to the pending claims" (Action at page 11, lines 18-19). Further, the examiner deems it relevant that Sabbadini teaches "other strategies that included a minicell that may express a protein such as invasi[]n to induce receptor mediated endocytosis" (*id.* at page 12, lines 5-6). Such a disclosure, according to the examiner, is presumed to be operable and enabled (*id.* at page 12, lines 11 and 14).

Applicant's argument relating to viruses and liposomes was intended to illuminate the conventional wisdom perceiving the incapability of minicells to deliver therapeutic nucleic acids

into cytoplasm and nuclei of a non-phagocytic mammalian cell. The examiner's characterization notwithstanding, therefore, this argument *is* relevant to the pending claims.

With respect to the examiner's statement regarding the presumed operability of Sabbadini's invasin-activated endocytosis approach, applicant would point out that their analysis does not turn on whether some aspect of the Sabbadini commentary is enabling. Rather, applicant has endeavored to show that the Sabbadini teachings about invasin-mediated endocytosis, read in context, would not have led the person of ordinary skill to the presently claimed invention. Indeed, as noted, those teachings would have directed the skilled artisan away from applicant's invention.

The examiner also contends that Sabbadini discloses that the minicell-based delivery method "results in transfer of the molecule from the interior of a minicell into the cytoplasm of the target cell (see column 24, line 22, column 165, lines 5-10)" (*id.* at page 7, lines 21-22). Applicant's deconstruction of these two passages above demonstrates, however, that Sabbadini envisages the release only of compounds and proteins but not of nucleic acids, which are much larger.

3. *Claims 36 and 37 manifest additional surprising aspects of the claimed invention*

As explained above, the claimed invention manifests, *inter alia*, the inventors' unexpected findings that (1) non-phagocytic mammalian cells can take up intact, bacterially derived minicells via tME, their large size (approximately 400 nm in diameter) aside, and (2) therapeutic nucleic acids, upon release from minicells degraded in the late-endosomal compartment, can escape and undergo expression, to therapeutic effect, in cellular nuclei. It is only by virtue of these and other insights that the present inventors could establish that effective minicell-mediated gene delivery to the nucleus of non-phagocytic cells is a function of copy number in a minicell (see application at page 10, second full paragraph).

Yet another surprising aspect of applicant's invention, therefore, is the fact that "effective minicell-mediated recombinant gene delivery to the nucleus of non-phagocytic cells relates to the number of plasmid copies carried by a minicell." *Id.* More specifically, a high copy number (*i.e.*, over 60 per minicell) gives rise to the highest efficiency compared to

median copy number (11 to 60) or low copy number (1 to 10). *Id.* In terms of unexpected results, therefore, claims 36 and 37 are patentable in their own right over the art of record.

III. Response to Double-Patenting Rejections

The examiner provisionally rejects the claims 1 and 15 under obviousness-type double patenting over claims 6 and 7 of each of U.S. applications No. 10/492,301 and No. 12/019,090. Applicant respectfully traverses.

It is well-established that the "same type of analysis is used for an obviousness-type double patenting inquiry as for a Section 103 obviousness inquiry." *The Procter & Gamble Co. v. Teva Pharmaceuticals USA, Inc.*, 566 F.3d 989, 90 USPQ2d 1947 (Fed. Cir. 2009). As provided above, the cited art fails a Section 103 test even it is accepted, *arguendo*, that it discloses a bispecific ligand that has a specificity for a mammalian cell surface receptor and is capable of activating rME. Since neither the '301 nor the '090 application even discloses this much, it necessarily follows that the respective double-patenting rejections based on these applications must fail.

1. The claimed invention is not obvious over the '301 claims

Applicant notes that claims 6-7 of the '301 application are directed to a method that includes contacting minicells with non-phagocytic mammalian cells. These claims, however, do not recite a bispecific ligand that has a specificity for a mammalian cell surface receptor and is capable of activating rME. As explained above, the cited art likewise does not disclose such a bispecific ligand either. Accordingly, claims 1 and 15 of the present application are not obvious over claims 6-7 of the '301 application.

2. The claimed invention is not obvious over the '090 claims

Similar to the '301 application, claims 6 and 7 of the '090 application do not recite a bispecific ligand that has a specificity for a mammalian cell surface receptor and is capable of activating rME, as the present claims require. As explained above, the cited art likewise does not disclose such a bispecific ligand either. Accordingly, claims 1 and 15 of the present application are not obvious over claims 6-7 of the '090 application.

From the foregoing, it is apparent that the present claims are not obvious over any claim of the cited applications. Withdrawal of the non-statutory obviousness type double-patenting rejection is warranted, therefore.

IV. Information Disclosure Statement

Applicant's representative received a letter, dated February 26, 2010, from Ryan E. Melnick under the letterhead of Knobbe Martens Olson & Bear LLP, the agent named on U.S. Patent No. 7,183,105 to Sabbadini *et al.*, presently cited. Accompanying the letter were nine publications that, Mr. Melnick alleged were material to the subject application. The letter and the publications are submitted concurrently with the supplemental information disclosure statement.

Applicant provides the following in response to the Melnick letter. The last part of the letter addresses commonly assigned, co-pending application serial No. 11/211,098. As these statements relate only to the question whether the art expected that a tumor cell could repeatedly engulf minicells through rME , they do not concern the patentability of the present claims. Therefore, these statements are not addressed here.

A. Hale *et al.* does not describe that minicells entered HeLa cells via receptor-mediated endocytosis

The first part of the Melnick letter relates to the subject application and a co-pending application serial No. 10/581,990. In the paragraph bridging pages 1 and 2, Mr. Melnick stated:

> [y]ou have argued that skilled artisans would have no reason to expect that particles as large as intact bacterially derived minicells of at least 400nm in diameter could be readily taken up by non-phagocytic mammalian cells via receptor-mediated endocytosis. However, Hale *et al.* (1983) discloses that bacterial minicells with a size range of about 400 nm in diameter are endocytosed into HeLa cells (a non-phagocytic human cervical cancer cell line) when proteins are present on the surface of minicells that are capable of stimulating receptor-mediated endocytosis. Specifically, Figures 4A and 4B (Hale 1983) depict minicells from *Shigella* that contain specific endocytosis-inducing outer membrane proteins encoded on and produced from the virulence plasmid of *Shigella flexneri* serotype 5 being repeatedly trafficked into HeLa cells (see description on page 346, column 1).

(Emphasis added.)

This statement is incorrect. Nowhere does Hale *et al.* describe a receptor-mediated endocytosis. Nor does the Hale publication mention a specific, endocytosis-inducing outer membrane protein, as Mr. Melnick alleges.

By way of introduction, the present inventors made the unheralded discovery that intact, bacterially derived minicells can be engulfed by a non-phagocytic mammalian cell via a receptor-mediated endocytosis process. Before this surprising discovery, it was well recognized in the art that large particles like minicells enter a non-phagocytic mammalian cell through an active and virulent invasive process. Such an active invasion may involve major molecular invasion proteins such as internalinA (InlA) and internalinB (InlB).

By contrast, receptor-mediated endocytosis is a passive and avirulent process. Exemplary such receptor-mediated endocytosis are clathrin-mediated endocytosis and caveolin-mediated endocytosis. See, generally, Doherty and McMahon, *Annu. Rev. Biochem.* 78: 31.1 – 31.46 (2009) (copy submitted).

At the outset, Hale *et al.* states clearly that the goal of the study was to characterize the role of plasmids in their contribution to the virulent phenotype of natural bacteria, motivated by the observed association of plasmids' presence in bacteria and the bacteria's virulent phenotype. (see page 340 in column 2, lines 1-7). In this context, Hale *et al.* indicates that the subject of the study was "the association of extrachromosomal elements with virulence in invasive enteric pathogens." *Id.* at page 340, column 2, lines 8-10 (emphasis added). Anucleate minicells, which contained such extrachromosomal elements, *e.g.*, plasmids, were used for this purpose. *Id.* at page 340 in column 2, lines 17-20.

Apparently, Hale's "minicells" were useful because they did not contain chromosomes so that the invasive enteric virulent phenotype could only be attributed to the plasmids. Thus, the process for the plasmid-containing minicells to enter host cells was expected to be the same as that for natural bacteria, *i.e.*, active and virulent invasion.

Not surprisingly, Hale *et al.* reports that these minicells entered HeLa cells through an active and virulent invasive process, just like how a natural bacterium enters a non-phagocytic mammalian cell. Nowhere in the entire article does Hale *et al.* state or hint that the minicells

entered the HeLa cells through a different mechanism, or in particular, the receptor-mediated endocytosis process, as Mr. Melnick claimed it did.

The section heading on the bottom of page 345, second column, and the immediately following paragraph reflect the understanding of Hale and his co-authors about this process:

> **Invasion of HeLa cells by *Shigella* minicells.** The study of plasmid-encoded virulence determinants in purified minicells was based on the assumption that these anucleate bacterial cells retain the virulence phenotype of vegetative parental cells … Figure 4B shows a higher magnification of the process of minicell invasion … Figure 4C shows minicells … which had lost the … plasmid did not establish intimate contact with the plasma membrane. This behavior is consistent with the avirulent phenotype of the vegetative cells of this strain.

Id., paragraph bridging pages 345 ad 346 (emphasis added).

Thus, Hale *et al.* confirms the author's perception that the process through which the plasmid-containing minicells entered HeLa cells was the same invasive and virulent process by which the parental cells accessed host cells. The publication also makes it clear that that plasmid-less minicells could not initiate such a process. To the contrary, the present inventors were the first to demonstrate that plasmid-less minicells can enter such mammalian cells, through a receptor-mediated endocytosis process. Thus, it is clear that the process observed in Hale *et al.* is different from receptor-mediated endocytosis, contrary to Mr. Melnick's statement.

In reference to Hale *et al.*, Mr. Melnick repeatedly employs the phrase "receptor-mediated." Yet, Hale *et al.* neither includes that phrase nor advances a similar concept. Not surprisingly, Mr. Melnick failed to pinpoint any specific text in Hale *et al.* to substantiate his statements in this regard.

Mr. Melnick also relied on Pal *et al.* and Watarai *et al.* to support the proposition that the invasive process observed in Hale *et al.* is receptor-mediated endocytosis, induced by the outer membrane protein encoded by the plasmids. Yet, both Pal *et al.* and Watarai *et al.* describe bacterial invasion, a process wholly distinct from receptor-mediated endocytosis. For example, see Pal *et al.* at page 2580, first column, line 1, and Watarai *et al.*, page 991 (summary), line 6. Further, Pal *et al.* and Watarai *et al.* describe how plasmid-containing bacteria adhered to the

mammalian cells through Ipa invasin-integrin binding. Unlike the situation with clathrin and caveolin, the binding of an invasin to integrin triggers virulent invasion rather than receptor-mediated endocytosis. It is apparent, therefore, that the processes disclosed in Pal *et al.* and Watarai *et al.* are not receptor-mediated endocytosis.

There is an extensive body of prior research papers and review articles that illuminates the active and virulent invasive process, through which bacteria attack mammalian cells. Hale *et al.* does not distinguish itself from this literature at all. Therefore, Mr. Melnick's reliance on Hale *et al.* to show minicell-induced, receptor-mediated endocytosis is simply a misreading of the prior literature.

B. Hale *et al.* and Jaffe *et al.* do not show that minicells are about 400nm in diameter

With reference to the present inventors' status as the first to determine that intact, bacterially derived minicells generally have a diameter of approximately 400nm, Mr. Melnick argued that:

> …[t]he scale reference bar in the Figures 4A and 4B (Hale 1983) micrographs clearly indicates that the minicells are about 400nm in diameter. In addition, Jaffe *et al.* (1988) teaches that *E. coli* minicells are about 400nm in diameter (see Figure 2) as do many other references …

(Page 2, second paragraph.) So stating, Mr. Melnick then asserted that applicant's submission concerning the inventors' discovery was wrong. Again, this assertion is itself erroneous.

First, Jaffe *et al.* in Figure 2 shows a size distribution graph of minicells. The sizes, based on the graph, range from about 250 nm to about 1500 nm. Jaffe *et al.* states that:

> … [t]he size distribution of anucleate cells measured after DAPI staining, including spherical and rod-shaped anucleate cells, was broader than expected, although no anucleate filaments were detected (Fig. 2).

Page 3096, second column, lines 3-6, emphasis added. Again,

> …[w]e observed that the *min* mutants spontaneously produced two types of anucleate cells, spherical minicells and anucleate rods. Both types of anucleate cells had a broad size

> distribution (Fig. 2). This was particularly surprising in the case of minicells, since cell diameter is constant in our experimental conditions.

Id. at page 3099, first column, lines 5-10, emphasis added.

It cannot be clearer, therefore, that Jaffe *et al.* actually discloses that the "minicells" under discussion had a broad range of sizes, in contravention of Mr. Melnick's statement.

With respect to the relevant figures in Hale *et al.*, applicant notes that Figure 4A, while lacking a reference scale bar *per se*, is clear in its indication that the "minicells" under discussion were characterized by a broad size range. This fact is not contradicted by Figure 4C, which Mr. Melnick fails to mention. Thus, Figure 4B does show three "minicells" that are about 400 nm in diameter, but this does not negate the teaching of other figures, which show wide ranging "minicell" sizes. Certainly, nothing in any of the Hale figures could have suggested that intact, bacterially derived minicells actually are uniformly sized, as the present inventors discovered.

Again, therefore, Mr. Melnick is incorrect in his statement about what the art understood concerning the size of minicells.

SUMMARY AND CONCLUSION

When read as a whole and in the context of contemporaneous understanding, Sabbadini at most would have conveyed, in general terms lacking any validating data, an approach for delivering material by way of an enclosing "minicell," a rubric expressly defined to include a diversity of cell derivatives, preferably denuded. According to Sabbadini, some manner of "minicell" presents or is attached to (i) a ligand that targets a host cell and (ii) another ligand, such as an invasin, that induces cellular uptake. Thus understood, Sabbadini does not teach every recited feature of the present claims but rather puts forward a number of genera – "minicells," cell-targeting function, nature of target cell, and uptake process, among others -- from which the knowledgeable reader may pick and choose, with little guidance from the reference itself. As a matter of law, therefore, Sabbadini does not qualify as an anticipating reference with respect to applicant's claimed invention.

Furthermore, Sabbadini's disclosure that a separate ligand, such as an invasin, should induce cellular uptake would have taught away from the presently claimed invention, which

entails the use of a bispecific ligand embodying not only a specificity for a surface receptor on the target, non-phagocytic mammalian cell but also a capability to activate rME uptake to the late-endosome compartment of that cell. In this regard the skilled artisan would have understood, too, that an invasin-induced cellular uptake, à la Sabbadini, is an active invasive mechanism that avoids the late-endosome/lysosome pathway, contrary to applicant's invention. Accordingly, no principled combination of Sabbadini with the cited secondary references can establish a *prima facie* case of obviousness.

In addition, applicant has underscored several aspects of the claimed invention, validated by experimental data from art-accepted models, that the person of ordinary skill would have found wholly unexpected, given the teachings discussed above. That those aspects were the subject of acclaim ("very interesting," "convincing," "unique," etc.) by independent experts, as evidenced above, further substantiates the non-obviousness of the claimed invention.

Applicant submits, therefore,that the application is in condition for allowance, and an indication to this effect is requested. Examiner Singh is invited to contact the undersigned directly, should he feel that any issue warrants further consideration.

Respectfully submitted,

Date 1 September 2010

By _____

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